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# The gut microbiota and host innate immunity: Regulators of host metabolism and metabolic diseases in poultry?[1]

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**Primary Audience:** Poultry Nutritionists, Poultry Immunologists, Poultry Veterinarians, Researchers

## SUMMARY

The endogenous intestinal microbiota represents the multitudes of microbes residing in the intestine and is integral in multiple physiological processes of the host, including being a key factor involved in host metabolism, BW, and energy homeostasis. The gut microflora, together with other environmental factors such as diet and stress, can play a central role in both immune and nutritional physiological balance. The immune response and nutrient metabolism are 2 fundamental biological systems indispensable to maintaining and preserving life. Each of these systems is capable of modulating the activity of the other to ensure that the host animal is capable of coordinating the appropriate responses under any conditions. Thus, metabolic systems are integrated with pathogen-sensing and immune responses, and these pathways are evolutionarily conserved. Several important networks sense and manage nutrients and integrate with immune and inflammatory pathways to influence the physiological and pathological metabolic states. For example, the Toll-like receptors family of the innate immune system, found on immune cells, intestinal cells, and adipocytes, recognize specific microbial components (e.g., lipopolysaccharides, lipoproteins, nucleic acids, and so on) and can sense nutritional signals, such as elevated glucose levels and saturated fatty acids. Likewise, metabolism-signaling pathways, such as leptin and other hormones, can also regulate immune functions. Thus, any immune alteration, specifically inflammation, can cause disturbances in host metabolism. Gut microbiota have evolved with the host as a mutualistic partner, but dysbiosis in the form of altered gut microbiome and gut microbial activities, as well as environmental factors including stress, may promote the development of metabolic disorders of poultry. Using mammalian studies as the experimental models, this review will provide evidence to hypothesize that intestinal dysbiosis or recognition of nutrient-derived factors (fatty acids and glucose) by the avian intestinal innate immune system could activate signaling pathways that affect the avian gut microbiota and induce the dysfunction of the integrated immune and nutritional metabolic systems that could be responsible for initiating many metabolic disorders of poultry.

**Key words:** gut microbiota, metabolism, nutrition, avian immunity, inflammation

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## DESCRIPTION OF PROBLEM

The gut is a multifaceted ecosystem with 3 primary interconnecting elements: (1) the intestinal epithelium with its neuroendocrine connections, (2) the immune system, and (3) the commensal microbiota [1]. These different elements have developed several bidirectional interactions. The gut microbiota regulates multiple host physiological pathways that form a collective host microbiota signaling, metabolic, and inflammatory alliance that connects the intestine, liver, muscle, and brain [2]. There is solid evidence from mammalian studies that the microbiome programs host immunity and drives a metabolome that affects energy balance and BW in the host [3]. In turn, the host immunity shapes the microbiome and host nutritional status influences elements of host defenses and makeup of commensal microbial community [1–4]. Further, the close evolutionary connection between the immune and metabolic responses suggest that both dietary metabolites and microbial components are often inducers of a chronic inflammatory response leading to profound and diverse metabolic diseases including obesity, type 2 diabetes, atherosclerosis, fatty liver disease, and stroke [5–7]. This raises the question of whether similar mechanisms mediate metabolic diseases of poultry [8, 9]. The etiology and pathogenesis of metabolic diseases in poultry is not clearly understood, but has been attributed, at least in part, to intense genetic selection for fast growth resulting in an inefficient cardiovascular system. Nevertheless, metabolic disorders in poultry can be attributed to such factors as nutritional excess, rapid growth, high nutrient intake, stress, infectious agents, and toxins [reviewed in 9]. Therefore, with no published data in poultry, it is reasonable to assume that metabolic diseases result from a multifactorial condition that includes changes in the gut microbiota (dysbiosis [1, 10]), over-nutrition that triggers increased inflammation [5, 6], and changes in nutrient metabolism, specifically lipids and glucose [5, 11], as has been found in mammals.

## THE GUT MICROBIOME

The complexity of the nutritional interactions within an animal is made substantially greater by the fact that animals play host to entire communities of commensal and symbiotic microbes that receive their nutrition from the host and, in turn, contribute essential nutrients and play a role in immune defense [12]. This second genome of vertebrates, the gut microbiota, has been shown to have profound and unanticipated effects on immune defense and inflammatory responses [10, 13]. Increasing evidence shows that the nutritional value of food is influenced by the structure and operation of the gut microbial community, and that food, in turn, shapes the microbiota and its vast collection of microbial genes (gut microbiome [10, 13]). Therefore, to define the nutritional value of foods and nutritional effects on host immunity, we need to know more about gut microbial communities as well as the avian mucosal immune system, how components of the microbiota affect mucosal immunity, and about how the metabolism of foods consumed by the gut microbial community affects the avian mucosal immunity.

The intestinal microbiome provides its host with crucial physiological functions that host organisms have not developed themselves [13–16]. Microbial metabolism increases energy yield and storage from the diet, regulates fat storage, and generates essential vitamins. This is primarily due to the fermentation of indigestible dietary polysaccharides [17]. Overall, the intestinal microbiota favor the renewal and barrier function of the gastrointestinal epithelium [13, 18] and have significant effects on host energy, gene expression, cell differentiation, and xenobiotic (molecules from outside the host that enter through the diet or produced by the microbiota) metabolism.

The chicken gastrointestinal tract is home to an ecosystem rich in microbial biodiversity, playing home to ≥500 phylotypes or ~1 million bacterial genes, which equates to 40 to 50 times the number in the chicken genome [19–23]. In fact, most bacteria (>80–90%) in the chicken cecum have never been cultured in the laboratory and are accessible only through molecular-biological approaches [21, 24]. These bacteria play important roles in the assimilation of nutrients from food, particularly through the release of energy from dietary fiber [21]. Thus, the gut microbiome is not a silent organ or simply a collection of passenger microorganisms; rather, in-

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testinal microbial communities represent active participants in vertebrate immunity and physiology [20–23].

Unlike the host genome, which is rarely manipulated by xenobiotic intervention, the microbiome is readily changeable by diet, ingestion of antibiotics, infection by pathogens, and stress [25–27]. The plasticity of the microbiome has been implicated in numerous disease conditions resulting from an unfavorable alteration of the commensal structure of gut microbiota [28, 29]. Dysbiosis has a potential 2-fold effect on host metabolism: (1) altering the ratio between beneficial gut bacterial species and detrimental gut bacterial species [22, 30–32], thus affecting the host's ability to harvest energy from food and to respond to energy intake, and (2) increasing the quantities of circulating bacteria or bacterial products (lipopolysaccharide) derived from the microbiota that are recognized by the innate immune system [33–36], thus inducing a low-grade chronic inflammation.

## MUCOSAL IMMUNITY

The establishment of an endogenous microbiota in the intestine represents an evolutionarily conserved characteristic of invertebrate [37] and vertebrate life [38–40], including poultry [40].

### *Intestinal Homeostasis*

The intestine is constantly exposed to nonself-derived antigenic substances including food antigens, invasive and noninvasive pathogens, and environmental toxins. In addition, as mentioned previously, the intestine is the home to $10^7$ to $10^{12}$ bacteria (poultry [30]). Thus, the intestine is exposed to an extraordinary antigenic load at any one time. Therefore, the intestinal tract is also an active immunological organ with more resident immune cells than anywhere else in the body [41]. In this antigenic environment, the various immune cells exist in a controlled equilibrium without causing apparent pathology (homeostasis [42]).

Immune responses at the intestinal mucosa are tightly controlled to remain tolerant of the commensal microbiota while concurrently maintaining the capacity to respond appropriately to harmful insult [10, 28, 29, 39]. Several cellular and molecular mechanisms have evolved that contribute to this remarkably effective balance (homeostasis) while averting detrimental overreactions that could damage the intestinal tissues or alter metabolic functions of the microbiota (dysbiosis; Table 1) [28, 29]. Thus, in general, the function of the intestinal immune system for maintaining homeostasis while mounting protective immunity to pathogens is manifested by (1) limiting direct bacterial contact with the epithelium and (2) rapid detection and removal of pathogens that penetrate the epithelium.

### *Limiting Direct Contact*

The intestinal epithelial cells contribute to the mucosal immunity [19]. Only 1 single layer of epithelial cells separates the densely colonized and environmentally exposed intestinal lumen from the largely sterile subepithelial tissue. The epithelium has evolved to maintain homeostasis in the presence of the enteric microbiota. It also contributes to rapid and efficient antimicrobial host defense in the event of infection with pathogenic microbes. Both epithelial antimicrobial host defense and homeostasis rely on signaling pathways induced by innate immune receptors demonstrating the active role of epithelial cells in the host-microbial interplay. Enterocytes have been shown to express pattern recognition receptors (**PRR**), both extracellular Toll-like receptors (**TLR**) and intracellular NOD-like receptors (**NLR**) that sense conserved bacterial products. In a normal state, TLR and NLR remain relatively unresponsive to the myr-

**Table 1.** Characteristics of intestinal health versus intestinal dysbiosis

| Health | Dysbiosis |
|---|---|
| Diverse or abundant microbiota | Reduced microbial diversity |
| Health level of short-chain fatty acid production | Skewed short-chain fatty acid profile |
| Intact mucosal barrier | Disrupted mucosal barrier |
| No overt inflammation | Inflammatory response initiated |
| *Firmicutes*, *Bacteroidetes* dominant | Elevated *Enterobacteriaceae* |

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iad bacteria overlying the mucosa. In a state of infection, injury, or another assault, however, they initiate a cascade of events that contribute to the induction of inflammatory host response [43, 44]. This interaction of epithelial cells with professional immune cells illustrates their integrated function within the mucosal tissue. Specialized epithelial cells produce a double (inner and outer) mucus layer [45] and secrete antimicrobial proteins (defensins, cathelicidins, C-type lectins [46, 47]) that limit bacterial exposure to the epithelial cells. Production of IgA, produced by intestinal B cells, provides an additional protective layer from luminal microbiota [48–50].

### *Rapid Detection and Removal of Pathogens*

Occasional breaches of the intestinal protective barriers do occur. Innate microbial sensing, mediated through PRR on the intestinal epithelial cells, lamina propria dendritic cells, and macrophages, initiates various pathways that mediate microbial killing and activate adaptive cells while keeping the resident microbial community in check without generating an overt inflammatory response to it [1, 51–53]. Dendritic cells present antigens to naïve $CD4^+$ T cells in lymphoid organs, such as Peyer's patches and cecal tonsils (in chickens), where differentiation of $CD4^+$ T cell subsets (Th1, Th2, Th17) with characteristic cytokine and intestinal homing profiles occurs [54].

Intraepithelial lymphocytes and γδT cell receptor-expressing T cells are lymphocytes that are uniquely present in the mucosa. Of the γδT cells in the intestinal lamina propria, there are significant numbers of IL-17-producing T (Th17) cells [55] and regulatory T cells [56–58]. The accumulation and function of these mucosal leukocytes are regulated by the presence of intestinal microbiota. By regulating these immune cells, the intestinal microbiota enhances the mucosal barrier function and allows the host to mount robust immune responses against invading pathogens while simultaneously maintaining immune homeostasis. Indeed, when properly guided by the microbiota, the mucosal immune system maintains a state of nonresponsiveness (tolerance) to dietary antigens and harmless commensal microbes [59].

In addition, recent studies in mammals have revealed that specific commensal bacterial species have been linked to vital roles in the mucosal immunity of mammals, specifically inducing the accumulation of certain immune cell populations. The polysaccharide antigen of *Bacteroides fragilis*, a prominent member of the gut microbiota, promotes the expansion of splenic T-helper cells and regulates the Th1 or Th2 cytokine production, as well as restoring the splenic architecture to that of conventionally raised mice [60]. *Bacteroides thetaiotaomicron*, another ubiquitous microbiota species, affects host gene expression, resulting in several effects in several organ systems [60]. The presence of higher numbers of bacteria belonging to the phylum Bacteroidetes has been shown to be associated with the development of IL-17 producing T-helper cells [61]. Different *Lactobacillus* species, also important members of the gut microbiota, differentially activate dendritic cells, inducing them to produce different arrays of inflammatory cytokines, thus playing an important role in the modulation of the Th1, Th2, and Th3 balance. Moreover, *Lactobacillus*-stimulated dendritic cells proceed to activate natural killer cells, thus potentiating gastrointestinal immunity. Segmented filamentous bacteria are implicated in the induction of the intestinal IgA and activation of intraepithelial lymphocytes and induction of MHC class II expression on intestinal epithelial cells [26]. Unfortunately, it has not been determined at this time whether such immune-modulating bacterial species are present or functional in poultry. However, the complexity of the enteric microbiota of poultry have been shown to have a striking influence on the dynamics of T cell receptor repertoires in the resident gut avian T lymphocytes [62].

## LINKING IMMUNITY AND HOST METABOLISM

Mounting an immune response to infection or injury is bioenergetically costly in all animal species, from flies to humans, including chickens [6, 7, 63, 64]. Therefore, the need to maintain metabolic homeostasis vies with the requirements of protecting the host from pathogens [7] that presents the host with a basic

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physiological task. However, this task has been optimized by the integration and coevolution of immune and inflammatory responses with the regulation of metabolism [6]. The close relationship between metabolism and immunity is evident by the architectural arrangement of metabolic organs (liver, adipose tissue) in vertebrates where macrophages and other immune cells are in close proximity to metabolic cells, such as hepatocytes and adipocytes [7]. Both cell types also share many signaling pathways and modules, including PRR [11, 35, 63–69]. Toll-like receptors, specifically TLR2 and TLR4, and NLR (NLRP3) can also sense excess nutritional signals, such as saturated fatty acids, glucose and lipids [11, 35, 66, 68–71]. Likewise, leptin, adiponectin, and metabolic hormones are able to regulate immune functions [72, 73]. Therefore, cellular and tissue homeostasis is dependent on the integration of and crosstalk between immune responses and metabolic regulation [52, 66].

In humans, a link between obesity, type 2 diabetes, and atherosclerosis implicates elevated amounts of glucose, oxidized LDL, and free fatty acids in disease pathogenesis, potentially as triggers for the production of proinflammatory cytokines by macrophages [6, 7]. Macrophages, which are intricately involved in inflammatory signaling pathways, infiltrate adipose tissue to a greater extent in obese individuals [6, 51]. Adipose tissue is a major source of the cytokines IL-1β, IL-6, tumor necrosis factor-α , and IL-18, which all play key roles in chronic inflammation. Part of the link between metabolic imbalance and pathogenic inflammation is likely the result of a range of innate immune pathways that are activated in these disease states.

## NUTRIENT SENSING AND INFLAMMATION

Inflammation is the most basic component of the innate immune response to irritation, injury, or infection [65, 74]. Classically, inflammation is manifested by swelling, redness, pain, and fever (rubor, calore, dolor, and calor), but with the outcome being resolution to homeostasis [75]. Hotamisligil [6] recently defined a subclass of inflammation, described in the literature as chronic inflammation, as metainflammation (metabolically triggered inflammation; Figure 1) that differs from classical inflammation in that "this condition is principally triggered by nutrients and metabolic surplus" [6]. What makes metainflammation so intriguing is that the inducers (metabolites and nutrients) are recognized by the same pathogen-sensing systems and stimulate the same signaling pathways that are involved in classical, acute, infectious inflammation as described previously [6].

## INFLAMMATION-GUT MICROBIOTA INTERACTION: THE NEW PARADIGM IN METABOLIC DISEASES

Recent studies using conventional and germ-free mice have provided definitive proof on the contributions of the gut microbiota to the pathophysiology of metabolic diseases in organs outside the intestine [reviewed in 76]. Basically, the gut microbiota contribute to the regulation of energy metabolism by 3 mechanisms: (1) increase energy yield from the diet [32, 77, 78]; (2) increased fat storage by microbiota-mediated suppression of fasting-induced adipose factor that leads to the suppression of lipoprotein lipase inhibitor and, consequently, increased lipoprotein lipase activity, which promotes increased uptake of fatty acids and triglycerides in adipocytes [32, 77, 78]; and (3) microbiota decrease levels of phosphorylated 5′-adenosine monophosphate-activated protein kinase in muscle and liver, thus inhibiting fatty acid oxidation [77]. Therefore, depending on other environmental factors and diet, the gut microbiota can cause an increase in fatty acid storage by 2 independent, but complementary mechanisms: decreased intestinal

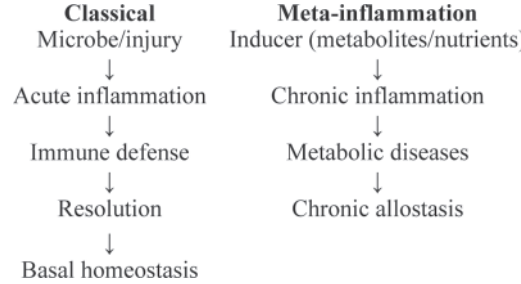


**Figure 1.** Comparison between classical inflammation and metainflammation.

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fasting-induced adipose factor and decreased muscle and liver AMP-activated protein kinase.

It is widely known that any changes in the microbes in gut microbiota (dysbiosis) and the regulation of mucosal and systemic host's immunity have been linked to different diseases, such as metabolic syndromes and associated disorders [79]. Most murine studies suggest that diet, especially high-fat diets, dramatically affects gut microbiota composition and leads to metainflammation and, consequently, metabolic diseases such as obesity and diabetes [6, 80]. The metabolic diseases have been shown to be associated with an increased expression of multiple proinflammatory cytokines (IL-6, tumor necrosis factor-α, IL-1β) in liver, muscle, and adipose tissue [81–83]. Further experiments demonstrated that LPS, derived from the gut microbiota, was the bacterial agonist that triggered the secretion of the cytokines via recognition by TLR4 [84]. Lipopolysaccharide is continuously produced in the intestine by the death of gram-negative bacteria and is carried into intestinal capillaries through a TLR4-dependent mechanism [84] and transported from the intestine to various tissues by lipoproteins synthesized by the intestinal epithelial cells in response to fat feeding [5, 85, 86].

## IMMUNITY-GUT MICROBIOTA INTERACTION AND METABOLIC DISEASES OF POULTRY?

There is little question that the gut microbiota and immune system interface is involved in systemic metabolic disturbances in humans, as described previously. Therefore, the obvious question is whether similar interactions of the gut microbiota and immune system interface also play a role in metabolic diseases in poultry?

Unfortunately, to date, there are few reports in the literature describing the direct interactions between the gut microbiota and immune response in poultry. Forder et al. [87] described a differential mucin profile and a greater numbers of goblet cells in the intestine of conventionally reared broiler chicks when compared with low bacterial load (isolator reared) broiler chicks. These mucosal immune changes were concluded to be due to the differential bacterial colonization of the respective chickens. Alterations in lymphocyte cell numbers and differences in intestinal lymphoid cellular subsets have been reported the intestines of germ-free chickens when compared with conventional chickens [88]. Mwangi et al. [63] found that the diversity of the gut microbiota have a striking effect on the complexity of T cell receptor repertoire on T cells in both the gut and the spleen.

The chicken has been found to have 11 known TLR [TLR1 (types 1 and 2), TLR2 (types 1 and 3), TLR3, TLR4, TLR5, and TLR7, as well as chicken-specific TLR 15, TLR16, and TLR21] [89, 90]. Likewise, recognition of microbial-associated molecular patterns by avian TLR activates the basic signaling pathways, as seen in mammals [91]. To date, there have been 4 NLR found on the chicken genome (NOD1–[92]; NLRP3–[93, 94]; NLRC5–[95–97]), and only 2 have been functionally described [95–97]; whereas 23 NLR genes have been found in mice and 23 proteins identified in humans [98]. However, there is no evidence in the literature that either avian TLR or NLR are nutrient-sensing receptors such as those seen in mammals. Further research is required to provide evidence that these receptors play a role in metabolic diseases of poultry.

Although the avian gut microbiota has been shown to have a considerable influence on poultry physiology, there have been virtually no studies on the interactive responses of the avian gut microbiota, inflammatory response, and metabolism [22, 23, 30, 99, 100]. Most studies dealing with the gut microbiota and immunity in poultry have concentrated on the effect of probiotics on protection against food-borne pathogens, such as Salmonella and Campylobacter [40, 101].

With these perspectives in mind, I asked the following question: Is there any evidence for a role of gut microbiota-host immune interface in avian metabolic diseases? To answer this question, I provide indirect, albeit circumstantial, evidence that points to the possibility that such interactions mediating lameness in broilers.

1. A recent study demonstrated that the gut microbiota is also a major regulator of bone mass in mice [102]. Germ-free mice exhibit increased bone mass associated with reduced number of osteoclasts per bone surface compared with conven-

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tionally raised mice [40]. Colonization of germ-free mice with a normal gut microbiota normalizes bone mass. Germ-free mice exhibited reduced expression of inflammatory cytokines in bone and bone marrow compared with conventional mice. Thus, increased bone mass is caused by fewer CD4 cells recirculating in blood and lymphoid tissue, resulting in a decreased frequency of CD4 T cells in bone marrow associated with a decreased expression of inflammatory cytokines and less osteoclastogenesis in the absence of gut microbiota.

2. Musculoskeletal disorders are considered metabolic diseases of poultry because of their adverse effects on performance [8]. Bacterial chondronecrosis with osteomyelitis is a major cause of lameness in poultry [103]. Bacteria reach the leg via the blood after translocation from the either gastrointestinal or respiratory tract [104, 105]. The bacterial translocation is probably due to dysbiosis induced by a general immunosuppression due to the stress response of being reared on wire flooring [105].
3. Wideman et al. [105], using a wire-floor model for inducing lameness, demonstrated that adding probiotics to the diet of the birds at 1 d of age significantly reduced the incidence of lameness.

Taken together, these results suggest a role for the gut microbiota and the metabolic disease of lameness in poultry. Could other metabolic diseases also be regulated by the gut microbiota? Further research will be required to prove or disprove this hypothesis.

## CONCLUSIONS AND APPLICATIONS

1. This review has detailed the general lack of information regarding the regulatory mechanisms of the gut microbiota and the functional changes on host physiology induced by the interactions occurring at the gut microbiota/intestinal interface.
2. Over the last 10 to 15 yr, an enormous body of work has been published on avian innate and adaptive immunity, detailing beneficial gut bacteria required for performance, and demonstrating the use of defined and undefined probiotics for competitive exclusion.
3. Using the mammalian literature as a model, my goal was to stimulate a discussion and interest in understanding on how these 2 physiological systems (gut microbiota and mucosal immunity) can interact to regulate or dysregulate the metabolism of modern poultry.
4. Meat-type poultry are constantly exposed to exogenous lipopolysaccharides both from the feed and from the local environment (used litter), thereby stimulating a metainflammatory state that could lead to metabolic disturbance of the cardiovascular and musculoskeletal systems.
5. Combining the pressures on modern poultry for rapid growth—high-nutrient-dense feed provided ad libitum leading to nutrient excess—with the potential for further inflammation due to nutrient sensing by PRR can resulti in altered metabolism.
6. More research is required to understand these initiating events that link inflammation to dysbiosis or nutrient excess to altered host physiology and the induction of metabolic diseases.

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